U.S. Application No.	International Application No.	Attorney Docket No.
UNKNOWN	PCT/NZ00/00159	**JAMES60.001APC**

Date: February 14, 2002

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 USC 371

International Application No.:	PCT/NZ00/00159
International Filing Date:	August 17, 2000
Priority Date Claimed:	August 17, 1999
Title of Invention:	RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE
Applicant(s) for DO/EO/US:	John West, Alan Leedham Hart and Owen A. Young

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. (X) This is a **FIRST** submission of items concerning a filing under 35 USC 371.

2. (X) This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Articles 22 and 39(1).

3. (X) A copy of the International Application as filed (35 USC 371(c)(2))

   a) (X) is transmitted herewith (required only if not transmitted by the International Bureau).
   b) () has been transmitted by the International Bureau.
   c) () a copy of Form PCT/1B/308 is enclosed.
   d) () is not required, as the application was filed in the United States Receiving Office (RO/US).

4. (X) International Search Report.

5. (X) Drawings in 5 pages.

6. (X) A FIRST preliminary amendment.
   () A SECOND or SUBSEQUENT preliminary amendment.

7. (X) International Application as published.

8. (X) A return prepaid postcard.

9. (X) The following fees are submitted:

				FEES
	BASIC FEE			$1,040
CLAIMS	**NUMBER FILED**	**NUMBER EXTRA**	**RATE**	
Total Claims	31 - 20 =	11 ×	$18	$ 198
Independent Claims	1 - 3 =	0 ×	$84	$ 0
	TOTAL FEES ENCLOSED			$1,238

10. (X) A check in the amount of $1,238.00 to cover the above fees.

The Commissioner is hereby authorized to charge only those additional fees which may be required, now or in the future, to avoid abandonment of the application, or credit any overpayment to Deposit Account No. 11-1410.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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CERTIFICATE OF MAILING BY "EXPRESS MAIL"

Attorney Docket No.	:	JAMES60.001APC
Applicants	:	West, et al.
For	:	RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE
Attorney	:	Mark R. Benedict
"Express Mail" Mailing Label No.	:	EL 001 480 515 US
Date of Deposit	:	February 14, 2002

I hereby certify that the accompanying

Transmittal; Demand; Copy of International Application in 18 pages; Drawings in 5 pages, Preliminary Amendment in 8 pages; International Search Report; Check for Filing Fee and a Return Prepaid Postcard are being deposited with the United States Postal Service "Express Mail Post Office To Addressee" service under 37 CFR 1.10 on the date indicated above and are addressed to the United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202.

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JAMES60.001APC PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	West, et al.	Group Art Unit Unknown
Appl. No.	:	Unknown	
Filed	:	Herewith	
For	:	RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE	
Examiner	:	Unknown	

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on

February 14, 2002
(Date)

Mark R. Benedict, Reg. No. 44,531

PRELIMINARY AMENDMENT

United States Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

Prior to examination on the merits, please amend the above-captioned patent application as follows:

IN THE SPECIFICATION:

On page 1, line 3, after the Title of the Invention please insert the following paragraph:

Cross Reference to Related Applications

This application is a National Phase application under 35 U.S.C. § 371 of PCT application PCT/NZ00/00159, filed August 17, 2000 and published in English, which claims priority to New Zealand application no. 337,276, filed August 17, 1999. The New Zealand application is incorporated herein by reference.

Appl. No. : **Unknown**
Filed : **Herewith**

IN THE ABSTRACT:

Please insert the abstract attached hereto, following the VERSION WITH MARKINGS TO SHOW CHANGES MADE, as page 18 of the application as filed.

IN THE CLAIMS:

Please amend the claims as follows:

2. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said method is performed in less than 30 minutes.

3. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said complex carbohydrate is selected from the group consisting of glycogen, lactate and a combination of these.

4. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said aqueous solvent is water.

5. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said aqueous solvent includes at least one agent to standardize ionic conditions for the method.

6. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein the formation of the homogenous slurry is effected with an apparatus selected from the group consisting of a high speed homogeniser, a low speed homogeniser, and an ultrasonic apparatus.

7. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said hydrolysing enzyme is selected from the group consisting of amyloglucosidase, α-amylase, α-glucosidase, and a combination thereof.

8. (AMENDED) A rapid method of measuring complex according to claim 7 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from the group consisting of a powder, a liquid suspension, and a solution.

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9. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said method further comprises a step (e): measuring the concentration of lactate in the sample.

10. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein steps (b) and (c) are performed simultaneously.

11. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein steps (c) and (d) are performed simultaneously.

16. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said glycogen measurement is effected by use of at least one sensor, each sensor incorporating said hydrolysing enzyme and glucose oxidase.

18. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said method is carried out at room temperature.

19. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 1 wherein said method is performed post-mortem, providing a measurement of concentrations of complex carbohydrates at the time of death.

20. (AMENDED) A rapid method of measuring complex carbohydrates according to claim 19 wherein said measurement is up to half an hour after slaughter.

21. (AMENDED) A method of measuring ultimate pH comprising the method of measuring complex carbohydrates according to claim 1 wherein said tissue is meat.

22. (AMENDED) A method of measuring ultimate pH comprising the method of measuring complex carbohydrates according to claim 1 wherein said tissue is muscle.

23. (AMENDED) A method of measuring ultimate pH according to claim 22 wherein said muscle is selected from the group consisting of the *longissimus lumborum*, *gluteus medius, semitendinosus,* and *longissimus lumborum* muscles.

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Please add the following claims:

24. (NEW) A rapid method of measuring complex carbohydrates according to claim 2 wherein said complex carbohydrate is selected from the group consisting of glycogen, lactate and a combination of these.

25. (NEW) A rapid method of measuring complex carbohydrates according to claim 3 wherein said hydrolysing enzyme is selected from the group consisting of amyloglucosidase, α-amylase, α-glucosidase, and a combination thereof.

26. (NEW) A rapid method of measuring complex carbohydrates according to claim 25 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from the group consisting of a powder, a liquid suspension, and a solution.

27. (NEW) A rapid method of measuring complex carbohydrates according to claim 25 wherein said glycogen measurement is effected by use of at least one sensor, each sensor incorporating said hydrolysing enzyme and glucose oxidase.

28. (NEW) A rapid method of measuring complex carbohydrates as claimed in claim 27 in which said sensor further comprises lactate oxidase.

29. (NEW) A method of measuring ultimate pH comprising the method of measuring complex carbohydrates according to claim 9 wherein said tissue is meat.

30. (NEW) A method of measuring ultimate pH comprising the method of measuring complex carbohydrates according to claim 9 in which said tissue is muscle.

31. (NEW) A method of measuring ultimate pH comprising the method of measuring complex carbohydrates according to claim 30 wherein said muscle is selected from the group consisting of *longissimus lumborum*, *gluteus medius, semitendinosus,* and *longissimus lumborum* muscles.

REMARKS

Amendments to the present application were made to place the application in accordance with practice before the United States Patent and Trademark Office and to more clearly claim the invention. No new matter is added herewith. Changes to the specification can be seen on a

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Filed : **Herewith**

separate page entitled VERSION WITH MARKINGS TO SHOW CHANGES MADE following the signature page. Deletions are in **[bold and brackets]** and insertions are underlined.

Conclusion

Should any issues arise which may delay prosecution of the present application the Examiner is respectfully invited to contact the under-signed attorney at the telephone number below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: 2/14/02

By: _____

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Appl. No. : **Unknown**
Filed : **Herewith**

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Deletions are in **[bold and brackets]** and insertions are underlined.

IN THE SPECIFICATION:

On page 1, line 3, after the Title of the Invention please insert the following paragraph:

Cross Reference to Related Applications

This application is a National Phase application under 35 U.S.C. § 371 of PCT application PCT/NZ00/00159, filed August 17, 2000 and published in English, which claims priority to New Zealand application no. 337,276, filed August 17, 1999. The New Zealand application is incorporated herein by reference.

IN THE ABSTRACT:

Please insert the abstract attached hereto, following the VERSION WITH MARKINGS TO SHOW CHANGES MADE, as page 18 of the application as filed.

IN THE CLAIMS:

Please amend the claims as follows:

2. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in]**according to claim 1 wherein said method is performed in less than 30 minutes.

3. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in either]**according to claim 1 **[or claim 2]**wherein said complex carbohydrate is selected from the group consisting of glycogen, lactate and a combination of these.

4. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said aqueous solvent is water.

5. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said aqueous solvent includes at least one agent to **[standardise]**standardize ionic conditions **[obtaining]** for the method.

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Filed : Herewith

6. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein the formation of the homogenous slurry is effected with an apparatus selected from the group consisting of**[:]** a high speed homogeniser**[;],** a low speed homogeniser, and an ultrasonic apparatus.

7. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said hydrolysing enzyme is selected from the group consisting of**[:]** amyloglucosidase**[;],** α-amylase**[;],** α-glucosidase, and a combination thereof.

8. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in]**according to claim 7 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from the group consisting of**[:]** a powder**[;],** a liquid suspension**[;],** and a solution.

9. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said method further comprises a step (e): measuring the concentration of lactate in the sample.

10. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein steps (b) and (c) are performed simultaneously.

11. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any on of claims 1 to 9]**according to claim 1 wherein steps (c) and (d) are performed simultaneously.

16. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said glycogen measurement is effected by use of at least one sensor**[s which incorporate],** each sensor incorporating said hydrolysing enzyme and glucose oxidase.

18. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said method is carried out at room temperature.

19. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said method is performed post-mortem, providing a measurement of concentrations of complex carbohydrates at the time of death.

20. (AMENDED) A rapid method of measuring complex carbohydrates **[as claimed in]**according to claim 19 wherein said **[method is performed wherein said]** measurement is up to half an hour after slaughter.

21. (AMENDED) **[Measurement of]**A method of measuring ultimate pH **[by use of]**comprising the method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said tissue is meat.

22. (AMENDED) **[Measurement of]**A method of measuring ultimate pH **[by use of]**comprising the method of measuring complex carbohydrates **[as claimed in any one of the preceding claims]**according to claim 1 wherein said tissue is muscle.

23. (AMENDED) **[Measurement of]**A method of measuring ultimate pH **[by use of the method of measuring complex carbohydrates as claimed in]**according to claim 22 wherein said muscle is selected from the group consisting of**[:]** the *longissimus lumborum***[;]**, *gluteus medius***[;]**, *semitendinosus*, and *longissimus lumborum* muscles.

TITLE: RAPID METHOD FOR MEASURING COMPLEX CARBOHYDRATES IN MAMMALIAN TISSUE

TECHNICAL FIELD

The present invention relates to a rapid method for measuring complex carbohydrates, particularly glycogen, in mammalian tissue. More particularly the present invention relates to the rapid measurement of glycogen in non-living mammalian tissue.

BACKGROUND ART

There are presently a number of methods of measuring complex carbohydrates, and particularly glycogen, in mammalian tissue. The discussion of these follows. The relevance of the measurement of glycogen includes, for example, the ability to use the results of glycogen measurement as a determination of the ultimate pH of meat. This in turn is a direct measure of many of the qualities of meat.

There are a number of known methods of measuring ultimate pH in meat: including use of liquid nitrogen in a freeze/thaw process and the use of a pH electrode for pH determination. There are a number of variations of this method, also. However the maintenance and use of liquid nitrogen in the quantities needed for the measurement on a continuous series of carcasses reveals hazards for the work environment. Also there is some doubt as to the accuracy and consistency of such measurement methods.

Methods of Measurement of Glycogen or Metabolites in Meat Samples

The iodine method: The principle of this method is that glycogen will react with a mixture of iodide, iodine and calcium chloride, forming an amber pigment in acid solution that has a linear absorption at least over a small, specified range. The glycogen is extracted from the meat with perchloric acid that is then filtered and centrifuged to recover a solution of glycogen which is reacted with the iodine. The extraction can also be by liquid nitrogen, potassium hydroxide, ethanol and ammonium chloride.

However, methods of extraction and then assay are time-consuming and employ aggressive chemical reagents.

Hydrolysis of glycogen with enzymes: The principle of this method is that glycogen hydrolyses to glucose, after which standard methods of measurement of free glucose may be used. The amyloglucosidase method of Dreiling et al (Meat Science, Vol 20, p. 167) is one such method, although other enzymes may be used. A muscle sample is homogenised with perchloric acid, and centrifuged, The supernatant, containing dissolved glycogen, is neutralised. Amyloglucosidase is added, converting the glycogen to glucose, for measurement. The first part of the method takes approximately 30 minutes at 37°C.

However there are some instances with the processing of meat in which a test of some 30 minutes or more is too long a time to wait for the test results, and some reagents are aggressive.

It is an object of the present invention to provide a rapid method for glycogen measurement in mammalian tissues, and in particular non-living tissue. It is a further object of the present

invention that in addition to the provision of a rapid accurate measurement of glycogen in tissue, the method also utilise mild or non-aggressive reagents.

Whilst the prior art and uses of glycogen measurement have been described with reference to a determination of the ultimate pH in meat, it will be appreciated that this method is not limited thereto. For example, low glycogen levels in the liver of bobby calves are an indicator of inadequate feeding before slaughter. Thus such rapid method of assessment of these levels could assist in ensuring good animal welfare.

It is an object of the present invention to address the foregoing problems or at least to provide the public with a useful choice.

Further aspects and advantages of the present invention will become apparent from the ensuing description which is given by way of example only.

DISCLOSURE OF INVENTION

For the purposes of this specification, the term "rapid" is used to refer to times of less than 30 minutes and, more preferably, significantly less than 30 minutes

According to one aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

(a) extracting a sample of tissue to be tested;

(b) forming a homogenous slurry of the sample with an aqueous solution;

(c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of

glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

Advantageously, said complex carbohydrate is glycogen. Advantageously, said aqueous solvent is water. Optionally said solvent may include at least one agent intended to standardise ionic conditions and/or facilitate the steps of the above method.

Preferably the formation of the homogenous slurry is effected with a high or a low speed homogeniser, or with an ultrasonic apparatus.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (b) and (c) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c) and (d) are performed simultaneously.

Advantageously, the hydrolysing enzyme may be any enzyme, or combination of enzymes, capable of hydrolysis of glycogen to glucose.

According to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in which said hydrolysing enzyme is selected from the group: amyloglucosidase; α-amylase; α-glucosidase, and a combination thereof.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above,

wherein the amyloglucosidase added in step (c) is in a form selected from: a powder; a liquid suspension; and a solution.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, in either embodiment, wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (d) and (e) are performed simultaneously.

According to another aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, wherein steps (c), (d) and (e) are performed simultaneously.

The measurement of both metabolites gives a good post-mortem estimate of the concentration of glycogen present in tissue at the time of death, no matter when the measurement is made.

Advantageously, the method is conducted at approximately room temperature, although it may be conducted at a temperature in the range 0°C to 100°C.

Measurement of Glucose

There are a range of methods for measuring glucose. For the present invention the most useful are those adapted from known technologies to measure glucose in blood. These are

usually based on the generation of hydrogen peroxide in stoichiometric proportion to glucose, as catalysed by glucose oxidase.

Therefore, according to a still further aspect of the present invention there is provided a rapid method of measuring complex carbohydrates in mammalian tissue, substantially as described above, and in which glycogen levels are measured and in which said measurement of the concentration of glucose is achieved by the construction of sensors incorporating said hydrolysing enzyme and glucose oxidase.

Measurement of Lactate

There are a range of methods for measuring lactate, including the standard NADH-linked method and those based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase. This latter compound can be incorporated into any sensor which may already incorporate glucose oxidase.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present invention will become apparent from the following example, which is given by way of example only, and with reference to the accompanying drawings in which:

Figure 1 is a graph of glucose concentration and glucometer reading of a glucose sample in acetate buffer;

Figure 2 is a graph of the glucometer reading for glucose and concentration of glucose

added to meat slurry samples;

Figure 3 is a graph of the kinetics of glucose formation from glycogen in acetate buffer in the present of amyloglucosidase;

Figure 4 is a graph of glucometer readings and the concentration of glucose added to a meat/acetate buffer slurry;

Figure 5 is a graph of glucose value and glycogen added to a post rigor meat slurry, at 5 minutes incubation at 55 °C;

Figure 6 is a graph of muscle glycogen concentration in post slaughter samples, using the method of measurement of the present invention;

Figure 7 is a graph showing the kinetics of glycogen loss, pH fall and lactate increase in a bovine muscle sampled after slaughter; and

Figure 8 is a graph of the repeatability of glycogen determination by the method of the present invention.

BEST MODES FOR CARRYING OUT THE INVENTION

Chemicals, Equipment and Meat.

Amyloglucosidase from the fungus *Aspergillus niger*, in powder form, was added to meat slurries as a liquid suspension or solution. In suspension, 25 mg amyloglucosidase was dissolved in 5 ml of 3.2M ammonium sulphate and adjusted to pH 6.0 with ammonia. This particular solvent is known to be one in which the enzyme is stable. As an alternative, a clear solution can be obtained by the use of 25 mg of powder, dissolved in 5 ml of 0.2M sodium

acetate at a pH of 4.5. α-D-Glucose of a standard analytical grade was used.

Glucose measurement was made with an Esprit glucometer (Bayer). Test sensors used in the Esprit were from Bayer New Zealand Limited. These were used for one reading only then discarded.

The meat samples tested were obtained from the *longissimus lumborum* muscle of a beef carcass, obtained from a butcher. Pre-rigor muscle, variously *gluteus medius*, *semitendinosus* and *longissimus lumborum*, was obtained from an abattoir. These muscles were dissected from unstimulated carcasses approximately 25 minutes after slaughter and tested very shortly thereafter. The muscles were held at room temperature while measurements were made.

Glycogen Test Procedure

The test medium for all experiments was 0.2M sodium acetate buffer at a pH of 4.4 and at a temperature of 55°C. In the experiments the muscle or meat (in samples of accurately known weight, but approximately 1 g) was homogenised in 5 ml of buffer, with a high speed Polytron shearing head. This was usually set at 25,000 rpm. Alternatively a lower speed homogeniser may be used, if so desired. The homogeniser may be a stainless steel paddle-like blade rotating at 2000 rpm in a steel cup within an interior shaped like a standard domestic Waring blender.

After homogenisation the enzyme solution was added. The volume of this solution was usually 200 µl, containing 1 mg of amyloglucosidase. The mixture was briefly shaken, then held at 55°C.

Small aliquots, of approximately 20 µl, were withdrawn at intervals with disposable pipettes,

and spotted onto plastic film. The glucometer sensor sampled these drops and returned a meter reading for glucose concentration in 30 seconds.

As a control, tests were also carried out with a range of glucose and glycogen concentrations in an acetate buffer to which no meat or meat samples had been added.

Lactate Measurement

In one range of experiments lactate concentration was also measured. At various times after slaughter, a crude aliquot of the slurry, containing homogenised muscle and amyloglucosidase was centrifuged in a micro-centrifuge (at 10, 000 rpm for 30 seconds). The clear supernatant was recovered and analysed for lactate concentration by the NADH-linked method.

Results

The results from the glucometer were in mg of glucose/dl. These results are tabulated in Fig. 1 of the attached drawings. It is noted that the relation was linear but that the readings were approximately double the glucose concentration in fact present and did not pass through the origin.

The exact reason for the approximate doubling of the readings is not known. However it is understood that this might relate to the characteristics of glucose in blood. α-D-glucose as a laboratory chemical dissolved in acetate at a pH of 4.5 may require a different calibration from that in blood. However, the failure of the straight line to pass through the origin

suggests that the acetate medium affected the sensor performance.

Example 1

Various samples of meat (the samples being as described above) between 0.90 and 1.16 g were homogenised in the high speed homogeniser in 5 ml of acetate buffer containing up to 16 mg of added glucose. This addition rate translates to approximately 267 mg/dl, assuming the density of meat is about 1 g/ml.

As can be seen from Fig. 2 of the attached drawings the relationship, the relationship between added glucose and the average meter reading is linear. The positive value of detected glucose with zero added glucose may be explained by the small quantities of glucose left over from glycolysis in the meat samples.

Example 2

The method of Example 1 was repeated over a range of samples and concentrations in which various quantities of glycogen (between 0 - 14 mg) were added to 5 ml of acetate buffer and the reactions were started by amyloglucosidase addition.

The results are as set out in Fig. 3 of the attached drawings, in which can be seen the glucose values peaked and declined slightly. The reason for the decline is not understood but it is possible that this may result from a contaminating activity in the enzyme preparation or from isomerisation reactions of glucose liberated from glycogen.

The data tabulated in Fig. 3 suggest that at even the highest concentrations of glycogen,

around 40 activity units of amyloglucosidase, are sufficient to fully hydrolyse the glycogen within approximately 5 minutes.

Example 3

The experiment the results of which are tabulated in Fig. 3 was repeated in the presence of rigor meat. Meat samples ranged from between 0.97 - 1.07 g in six tests. The results are as set out in Fig. 4 of the attached drawings.

Individual data points were abstracted to plot glycogen added against meter readings for 5 minutes after the amyloglucosidase addition. The results of this are shown in Fig. 5. A quadratic equation was fitted, but the shape was close to a straight line.

Example 4

Samples of *gluteus medius* and a *semitendinosus* muscle were removed from pre-rigor meat, cut to samples in the size between 0.95 - 1.05 g, and homogenised in 5 ml of acetate buffer. This slurry was then treated with amyloglucosidase. The tests began 1.3 hours after slaughter, first with the *semitendinosus* muscle and at that time the muscle contained only 3.8 mg glycogen /g. By 2 hours the glycogen level declined to 1 mg/g.

In contrast, *gluteus medius* muscle sample contained 13.1 mg/g at 2.3 hours post slaughter, and the value declined steadily with time. The results are as shown in Fig. 6.

This experiment was repeated using *longissimus lumborum* muscle from another animal. This muscle is frequently used as an indicator of high pH condition. Lactate concentrations

were also measured.

The above tests as described were carried out and the results are as shown in Fig. 7 of the attached drawings. In this method the measurement of lactate was as per the standard NADH-linked method rather than the use of a sensor based on lactate oxidase.

Example 5

The robustness of the preferred embodiment of the test of the present invention was further determined by the following: meter readings were recorded in which triplicate aliquots of glycogen were added to three replicate rigor meat samples weighing 1.0 +/- 0.05 g. The slurry was treated in the same manner as described above for Example 2. The results of this are set out in Fig. 8 of the attached drawings.

Whilst the Examples given above to show the best method of performing the invention are all with reference to meat samples (beef) it would be appreciated by those skilled in the arts that other tissue samples may be equally treated in like manner to produce glycogen measurement as a result.

Aspects of the present invention have been described by way of example only and it should be appreciated that modifications and additions may be made thereto without departing from the scope thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

CLAIMS

1. A rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of:

(a) extracting a sample of tissue to be tested;

(b) forming a homogenous slurry of the sample with an aqueous solution;

(c) adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and

(d) measuring the concentration of glucose in the slurry.

2. A rapid method of measuring complex carbohydrates as claimed in claim 1 wherein said method is performed in less than 30 minutes.

3. A rapid method of measuring complex carbohydrates as claimed in either claim 1 or claim 2 wherein said complex carbohydrate is selected from glycogen, lactate and a combination of these.

4. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said aqueous solvent is water.

5. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein said aqueous solvent includes at least one agent to standardise ionic conditions obtaining for the method.

6. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein the formation of the homogenous slurry is effected with apparatus selected from: a high speed homogeniser; a low speed homogeniser and an ultrasonic apparatus.

7. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said hydrolysing enzyme is selected from the group: amyloglucosidase; α-amylase; α-glucosidase, and a combination thereof.

8. A rapid method of measuring complex carbohydrates as claimed in claim 7 wherein said hydrolysing enzyme is amyloglucosidase which is in a form selected from: a powder; a liquid suspension; and a solution.

9. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method further includes a step (e): measuring the concentration of lactate in the sample.

10. A rapid method of measuring complex carbohydrates as claimed in any one of the

preceding claims wherein steps (b) and (c) are performed simultaneously.

11. A rapid method of measuring complex carbohydrates as claimed in any one of claims 1 to 9 wherein steps (c) and (d) are performed simultaneously.

12. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein steps (d) and (e) are performed simultaneously.

13. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein steps (c) to (e) are performed simultaneously.

14. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is by the NADH-linked method.

15. A rapid method of measuring complex carbohydrates as claimed in claim 9 wherein said measurement of lactate concentration is based on the generation of hydrogen peroxide in stoichiometric proportion to lactate, as catalysed by lactate oxidase.

16. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said glycogen measurement is effected by use of sensors which

incorporate said hydrolysing enzyme and glucose oxidase.

17. A rapid method of measuring complex carbohydrates as claimed in claim 16 wherein said sensor further includes lactate oxidase.

18 A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method is carried out at room temperature.

19. A rapid method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said method is performed post-mortem, providing a measurement of concentrations of complex carbohydrates at the time of death.

20. A rapid method of measuring complex carbohydrates as claimed in claim 19 wherein said method is performed wherein said measurement is up to half an hour after slaughter.

21. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said tissue is meat.

22. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in any one of the preceding claims wherein said tissue is muscle.

23. Measurement of ultimate pH by use of the method of measuring complex carbohydrates as claimed in claim 22 wherein said muscle is selected from: the *longissimus lumborum*; *gluteus medius; semitendinosus* and *longissimus lumborum* muscles.

ABSTRACT

The invention discloses a rapid method of measuring complex carbohydrates in mammalian tissue, said method comprising the steps of: extracting a sample of tissue to be tested; forming a homogeneous slurry of the sample with an aqueous solution; adding sufficient hydrolysing enzyme for ensuring complete hydrolysis of glycogen in the slurry; and measuring the concentration of glucose in the slurry. The method can be conducted post-mortem to assay the concentration at the same time of slaughter. The invention further discloses the measurement of lactate concentrations. The method of the invention can be conducted in thirty minutes or less.

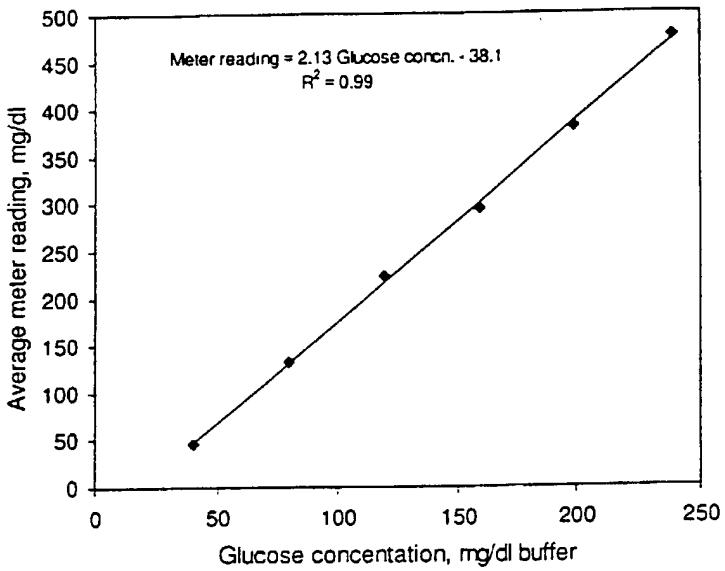

Figure 1

Relationship between the Esprit meter value for glucose and the concentration of α-D-glucose in acetate buffer at pH 4.5. Points are means of duplicates.

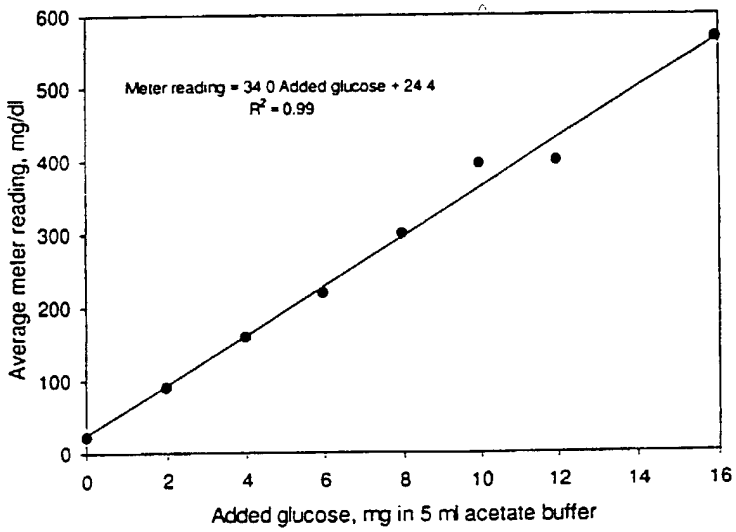

Figure 2

Relationship between the Esprit meter value for glucose and the concentration of α-D-glucose in a meat/acetate buffer slurry at pH 4.5. Points are means of duplicates.

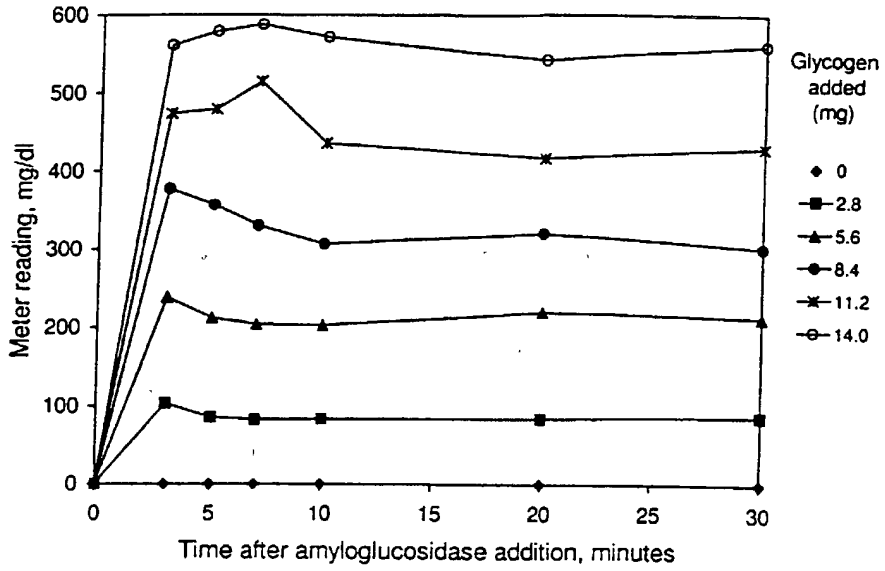

Figure 3
Kinetics of glucose formation from glycogen in acetate buffer in the presence of amyloglucosidase.

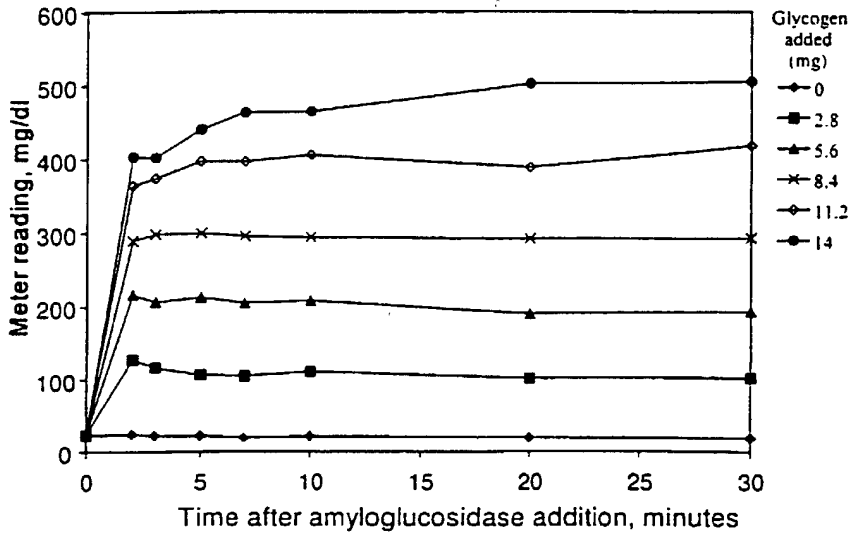

Figure 4
Kinetics of glucose formation from glycogen by amyloglucosidase in a meat slurry.

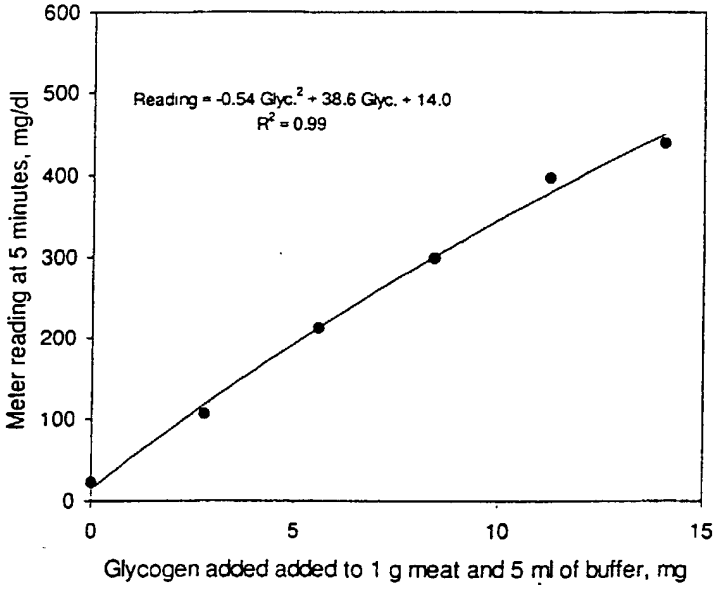

Figure 5

Relationship between the Esprit meter value for glucose and the quantity of glycogen in a post rigor meat slurry that also containing amyloglucosidase. Values were those recorded after 5 minutes incubation at 55°C. A quadratic equation was fitted to the data.

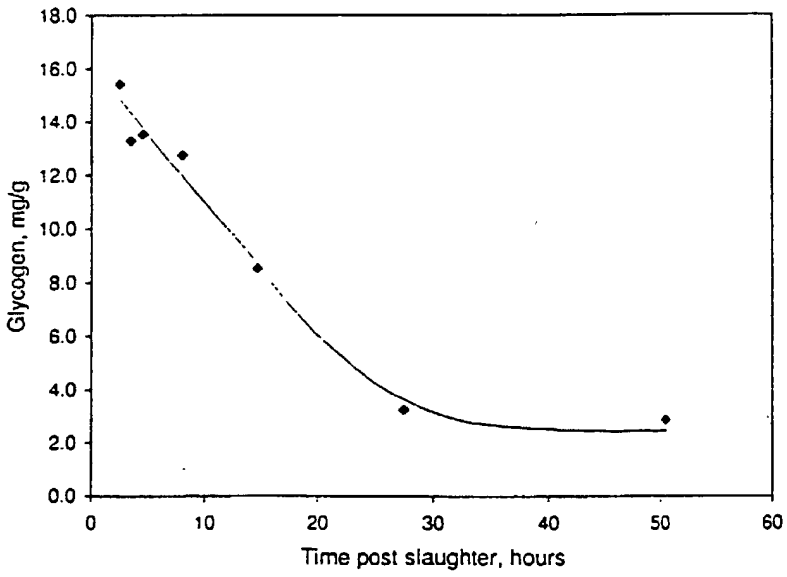

Figure 6

Kinetics of glycogen loss in *gluteus medius* as determined by the amyloglucosidase method.

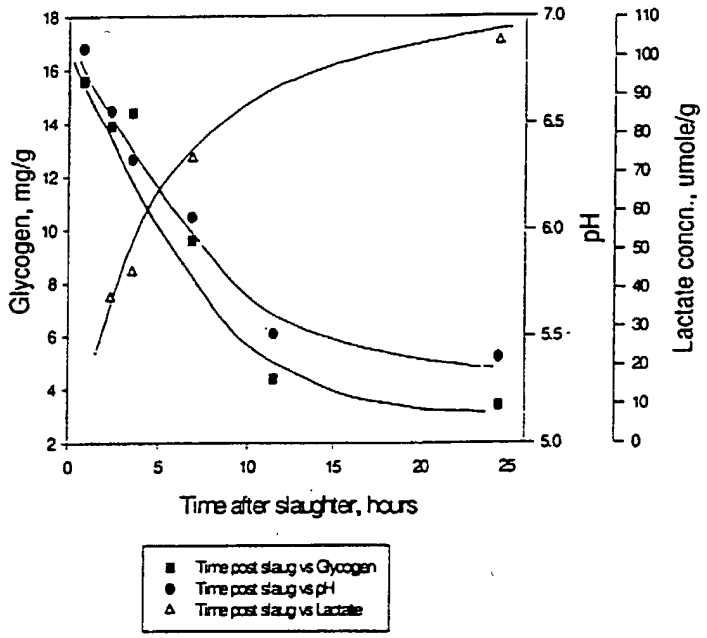

Figure 7

Kinetics of glycogen loss, pH fall and lactate increase in *longissumis lumborum* from an unstimulated carcass.

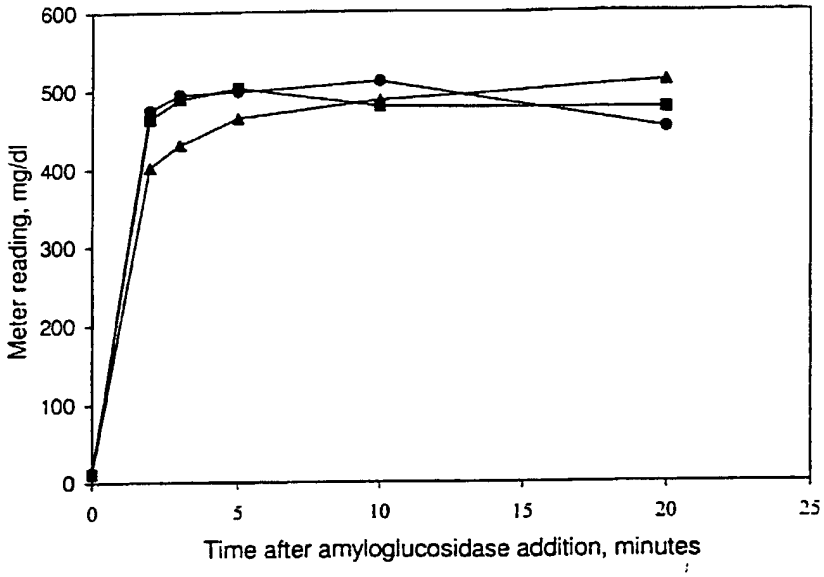

Figure 8

Repeatability of glycogen determination as glucose in the presence of rigor meat.

Rapid Method... Mammalian Tissue 10/069441 - 40196
-USA

DECLARATION AND POWER Of ATTORNEY

As a below named inventor, I hereby declare:

That my residence, post office address and citizenship are as stated below next to my name.

That I verily believe I am an original, first and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled: Rapid Method for Measuring Complex Carbohydrates in Mammalian Tissue

the specification of which (check one)

[ ] is attached hereto.
[√] was filed on 14 February 2002 as
Application Serial No. 10/069,441
and was amended on n/a
(if applicable)

That I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

That I acknowledge the duty to disclose information known to be material to patentability of this application in accordance with Title 37, Code of Federal Regulation, § 1.56(a).

That I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s) Priority Claimed

337276	New Zealand	17 August 1999	[√] [ ]
PCT/NZ00/00159	PCT	17 August 2000	[√] [ ]
(Number)	(Country)	(Day/Month/Year Filed)	**Yes** No

That I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulation, § 1.56(a) which became between the filing date of the prior application and the national or PCT International filing date of this application:

United States Application(s)

10/069,441	14 February 2002	pending
(Application Number)	(Filing date)	(Status -- patented, pending, abandoned)

That all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and agents, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith and request that all correspondence and telephone calls in respect to this application be directed to KNOBBE MARTENS OLSON & BEAR LLP, 620 Newport Center Drive, 16th Floor, Newport Beach, CA 92660, Telephone No. (949) 760 0404:

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1-00

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